



---

Year: 2017

---

## **Splenitis in 33 dogs**

Ferri, F ; Zini, E ; Auriemma, E ; Castagnaro, M ; Coppola, L M ; Peano, A ; Martella, V ; Decaro, N ; Kuhnert, P ; Ferro, S

**Abstract:** Splenitis is uncommonly reported in dogs. Herein, the authors describe its prevalence, clinical findings and outcomes, histologic patterns, and causes. Splenic samples of dogs diagnosed with splenitis between 2005 and 2013 were collected and stained with hematoxylin and eosin, Gram, green-Gram, Giemsa, periodic acid-Schiff, and Ziehl-Neelsen. Samples were processed for polymerase chain reaction (PCR) to detect bacteria, fungi, and protozoa (*Leishmania infantum*, *Hepatozoon canis*). Thirty-three of 660 splenic samples (5%) had splenitis. Clinical findings and outcomes were available in 19 dogs (58%); 49% had weakness, 33% had fever, and 84% survived. The most frequent inflammatory patterns included purulent splenitis (27%), pyogranulomatous splenitis (24%), and neutrophilic perisplenitis (15%). One dog had a putative diagnosis of primary splenitis; in 8 dogs, microorganisms were identified histologically or by PCR in the spleen without obvious comorbidities. Twenty-four dogs (73%) had concurrent diseases; a permissive role in the development of splenitis was suspected in 21 of these cases. Histologic examination identified the cause of splenitis in 10 dogs. Bacteria were identified by PCR in 23 cases, but the bacteria were confirmed histologically in only 6 of these. *Leishmania* was detected with PCR in 6 dogs. *Leishmania* was identified in 1 dog and *H. canis* in another histologically, but both were PCR negative. Fungi were identified in 8 spleens by PCR and in 1 by histology. This study suggests that splenitis is uncommon in dogs and is frequently associated with systemic diseases. Prognosis is favorable in most cases. Identification of bacteria, fungi, and protozoa in the spleens of affected dogs with PCR should be interpreted cautiously, because the findings are not confirmed histologically in many cases.

DOI: <https://doi.org/10.1177/0300985816653989>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-128092>

Journal Article

Accepted Version

Originally published at:

Ferri, F; Zini, E; Auriemma, E; Castagnaro, M; Coppola, L M; Peano, A; Martella, V; Decaro, N; Kuhnert, P; Ferro, S (2017). Splenitis in 33 dogs. *Veterinary Pathology*, 54(1):147-154.

DOI: <https://doi.org/10.1177/0300985816653989>

## **SPLENITIS IN DOGS: 33 CASES (2005-2013)**

F. Ferri<sup>1</sup>, E. Zini<sup>1,2,3</sup>, E. Auriemma<sup>1</sup>, M. Castagnaro<sup>4</sup>, L.M. Coppola<sup>2</sup>, A. Peano<sup>5</sup>, V. Martella<sup>6</sup>, N. Decaro<sup>6</sup>, P. Kuhnert<sup>7</sup>, S. Ferro<sup>4</sup>

<sup>1</sup>Istituto Veterinario di Novara, Strada Provinciale 9, 28060 Granozzo con Monticello (Novara), Italy (FF, EZ, EA). <sup>2</sup>Department of Animal Medicine, Production and Health, University of Padua, viale dell'Università 16, 35020 Legnaro (Padua), Italy (EZ, LMC). <sup>3</sup>Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland (EZ). <sup>4</sup>Department of Comparative Biomedicine and Food Sciences, University of Padua, Viale dell'Università 16, 35020 Legnaro (Padua), Italy (MC, SF). <sup>5</sup>Department of Veterinary Science, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco (Turin), Italy (AP). <sup>6</sup>Department of Veterinary Medicine, University of Bari, Strada Provinciale per Casamassima km 3, 70010 Valenzano (Bari), Italy (VM, ND). <sup>7</sup>Institute Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Länggass-Strasse 122, 3012 Bern, Switzerland (PK).

**Corresponding author:** Filippo Ferri

Mailing address: Istituto Veterinario di Novara, Strada Provinciale 9, 28060 Granozzo con Monticello (NO), Italy. Phone: +39-0321-4600155; Fax: +41-0321-4600101

**E-mail:** [filippo.ferri@istitutoveterinarionovara.it](mailto:filippo.ferri@istitutoveterinarionovara.it)

## Abstract

Splenitis is uncommonly reported in dogs. Herein, we describe its prevalence, clinical findings and outcome, histological patterns, and causes. Splenic samples of dogs diagnosed with splenitis between 2005-2013 were collected and stained with hematoxylin-eosin, Gram, green-Gram, Giemsa, periodic acid-Schiff (PAS), and Ziehl-Neelsen. Samples were processed for PCR to detect bacteria (16s rRNA), fungi (ITSs), and protozoa (*Leishmania infantum*, *Hepatozoon canis*). Thirty-three (5%) of 660 splenic samples had splenitis. Clinical findings and outcome were available in 19 dogs (57.6%); 48.5% had weakness, 33.3% fever, and 84.2% survived. The most frequent inflammatory patterns included purulent splenitis (27.3%), pyogranulomatous splenitis (24.2%), and neutrophilic perisplenitis (15.1%). One dog had a putative diagnosis of primary splenitis; in 8, PCR or histology identified microorganisms in the spleen without obvious comorbidities. Twenty-four dogs (72.7%) had concurrent diseases; a permissive role in the development of splenitis was suspected in 21 cases. Histology identified the cause of splenitis in 10 dogs; in 23, bacteria were identified by PCR but in only 6 with histology. *Leishmania* was detected with PCR in 6 dogs. Histology identified *Leishmania* in one dog and *Hepatozoon canis* in another, but both were PCR-negative. Fungi were identified in 8 spleens by PCR and in one by histology. This study suggests that splenitis is uncommon in dogs and is frequently associated with systemic diseases. Prognosis is favorable in most cases. Identification of bacteria, fungi, and protozoa in the spleen of affected dogs with PCR should be interpreted cautiously because histology does not confirm infection in many cases.

## Keywords

Dog, spleen, PCR, histology, inflammation, splenitis, prognosis.

## Introduction

The spleen is a secondary lymphoid organ as are lymph nodes and mucosa-associated lymphoid tissues (MALT). It is formed by the red and white pulp; the first, harbors numerous macrophages and dendritic cells that are part of the reticuloendothelial system, whereas the latter B and T lymphocytes. Both take part in the immune response and react to several pathologic insults, ranging from infectious to neoplastic diseases.<sup>50</sup> Non-neoplastic diseases of the spleen are commonly described in dogs; based on the current literature, their prevalence among all splenic diseases, ranges from 35 to 70%.<sup>6,9,10,19,46</sup> Amyloidosis, congestions, hemorrhages, hematomas, hyperplastic and low grade fibrohistiocytic nodules, infarctions, necrosis, siderotic plaques, splenitis, thrombosis, torsions, and traumatic ruptures are included among non-neoplastic disorders.<sup>46</sup>

Splenitis is an inflammatory process of the splenic parenchyma that is uncommon in dogs. Survey studies of splenic diseases in this species have reported a prevalence of splenitis ranging from 0.9% to 8%.<sup>6,19</sup> Among its causes, splenitis was described in only single case reports associated with fungal,<sup>2,4,13,24,33,44,45,51</sup> protozoan,<sup>3,17,28,38</sup> or bacterial infections.<sup>1,2,5,11,16,30,39,41,47</sup>

The cases related to fungal infections, invariably had poor prognosis. In these dogs, necropsy identified splenomegaly and multiple foci of necrosis while histology documented pyogranulomatous inflammation, multinucleated giant cells and, in some cases, fungal hyphae. Fungi that have been identified in dogs with splenitis are *Monocillium indicum*,<sup>24</sup> *Scedosporium prolificans*,<sup>13</sup> *Scopulariopsis chartarum*,<sup>51</sup>

*Aspergillus terreus*,<sup>33</sup> *Cryptococcus neoformans*,<sup>7</sup> *Candida albicans*,<sup>4</sup> *Ochroconis gallopavum*,<sup>45</sup> *Xylohypha bantiana*,<sup>42</sup> and *Acremonium spp.*<sup>44</sup>

The protozoa identified in dogs with splenitis include *Neospora caninum*,<sup>17</sup> *Hepatozoon canis*,<sup>3,15</sup> *Trypanosoma cruzi*,<sup>28</sup> and *Leishmania chagasi*.<sup>35,38,49</sup> *Leishmania chagasi* and *Leishmania infantum*, responsible of leishmaniasis in the New and Old World respectively, are considered genomically indistinguishable and therefore synonymous.<sup>55</sup> Lesions of the spleen of dogs with leishmaniasis are characterized by thickening of trabecular regions, granulomatous reaction and hemorrhagic areas, atrophy of lymphoid follicles and marginal zone, abundant macrophages with amastigotes in the cytoplasm, and perisplenitis.<sup>35,38,49</sup>

Considering bacterial splenitis, dogs with *Staphylococcus spp.*,<sup>1</sup> *Mycobacterium avium* and *tuberculosis*,<sup>5,16,25,30</sup> *Listeria monocytogenes*,<sup>41</sup> *Bartonella henselae* and *vinsonii*,<sup>39</sup> *Clostridium spp.*,<sup>2</sup> *Bacillus anthracis*,<sup>26</sup> *Burkholderia pseudomallei*,<sup>47</sup> and multiple bacterial infection<sup>10</sup> have been described; in these cases, inflammation of the spleen based on histology is generally pyogranulomatous<sup>5,39</sup> or neutrophilic.<sup>2</sup> Splenic abscesses, likely due to bacteria, have been described in some dogs.<sup>1,9,11</sup>

Interestingly, in one study it was shown that 35% of surgically-excised spleens were positive for bacterial culture, irrespective of the disease affecting the organ, and the most commonly identified bacteria include *Staphylococcus spp.*, *Enterococcus spp.*, and *Klebsiella pyogenes*.<sup>36</sup> Prognosis for bacterial and protozoan splenitis is generally better than that of fungal origin, although it may vary according to the causative microorganism and individual factors.

The aims of this study were to define the prevalence of splenitis in dogs, describe clinical findings and outcome, characterize histological patterns of splenic inflammation, and investigate possible causes.

## **Materials and Methods**

### *Dogs, clinical data, and questionnaire*

Splenic biopsies of dogs collected at necropsy or at surgery after splenectomy and partially or entirely submitted to the Department of Comparative Biomedicine and Food Sciences of the University of Padua between 2005 and 2013 with a histologic diagnosis of splenitis were retrieved from archives and included in the study. Age, sex, breed, and clinical data of affected dogs were recorded. Due to the retrospective nature of the study and because most dogs were not admitted at the authors' institutions, clinical data were often incomplete or entirely missing. Owners were therefore contacted to retrieve further information. In particular, a telephone-based questionnaire was proposed, with the following questions: i) what clinical signs did the dog present at admission?; ii) was the dog affected by other diseases?; iii) what was the final diagnosis, other than splenitis?; iv) what was the treatment of the dog after splenectomy?; v) how long did the dog survive following surgery?

### *Histology*

From each paraffin-embedded splenic sample previously fixed in 10% neutral-buffered formalin, 6 consecutive sections of 4  $\mu$ m were obtained and mounted on slides. The first section was stained with hematoxylin and eosin, and the others were stained with Giemsa, and/or periodic acid-Schiff (PAS), Ziehl-Neelsen, Gram, and green-Gram. All sections were mounted onto labeled glass slides with Eukitt (Bio-Optica, Milan, Italy). Sections were evaluated by light microscopy (Olympus BX-40, Milan, Italy) and findings were classified according to the type and localization of

inflammation documented, as described in Supplemental Table 1. Tissue sections were also examined to identify bacteria, protozoa, or fungi. All slides were assessed by two pathologists (SF, MC) and any difference in diagnosis was resolved by consensus.

### *Staining procedures*

#### Hematoxylin-eosin

Slides were stained for 7 min in hematoxylin (Merk Millipore, Darmstadt, Germany) and then were rinsed in running water for 5 min; after that, they were stained in eosin (Bio-Optica, Milan, Italy) for 1 min and dehydrated using alcohol solutions of increasing concentrations (50%, 70%, 90%, and 100%) and xylene.

#### Giemsa

The staining solution was prepared using 2.4 ml of citric acid 0.1 M (Sigma-Aldrich, Milan, Italy), 1.6 ml of 0.1 M disodium phosphate (Sigma-Aldrich, Milan, Italy), 10 ml of Giemsa solution (Sigma-Aldrich, Milan, Italy), and deionized water. Slides were placed in this solution for 60 min, rapidly decolorized with acetone, and placed in a 50% acetone-xylene solution and then xylene only.

#### Periodic acid-Schiff (PAS)

Slides were treated with 1% periodic acid (Sigma-Aldrich, Milan, Italy) for 10 min, rinsed in distilled water, and Schiff reagent (Labochimica, Padua, Italy) was added for 20 min. Sections were rinsed with running water and colored with Mayer's



hematoxylin (Merk Millipore, Darmstadt, Germany) for 2 min and then rinsed again. The final step was dehydration using graded alcohol solutions.

### Ziehl-Neelsen

Ten drops of periodic acid (Sigma-Aldrich, Milan, Italy) were added to tissue slices for 10 min and then rinsed with distilled water. The procedure was repeated using 10 drops of carbol-fuchsin (Merk Millipore, Darmstadt, Germany) for 30 min, 10 drops of acid buffer (Carlo Erba, Milan, Italy) of differentiation for 1 min, and 10 drops of Mayer hematoxylin (Merk Millipore, Darmstadt, Germany) for 2 min; between each step, slides were rinsed with distilled water.

### Gram

Crystal violet staining (Sigma-Aldrich, Milan, Italy) was added to sections for 1 min, and, after rinsing, the procedure was repeated with Lugol solution (Sigma-Aldrich, Milan, Italy). Sections were then treated with acetone followed by safranin (Sigma-Aldrich, Milan, Italy). Slides were stained using basic fuchsin (Merk Millipore, Darmstadt, Germany) and then placed in acetone and acetone-picric acid solutions (Carlo Erba, Milan, Italy).

### Green-Gram

Hucker-Conn solution (Sigma-Aldrich, Milan, Italy) was added to slides, followed by Langeron solutions (Sigma-Aldrich, Milan, Italy). The sections were then decolorized with acetone (Sigma-Aldrich, Milan, Italy), colored with fast green safranin (Sigma-

Aldrich, Milan, Italy), rinsed with 100% ethanol (Sigma-Aldrich, Milan, Italy), placed in a 1% solution of acetic acid alcohol (Carlo Erba, Milan, Italy), and rinsed again with ethanol.

#### *DNA extraction*

Total DNA from splenic samples was purified using a commercial kit (NucleoSpin Tissue; Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Briefly, 2 slices of 10 µm of paraffin-embedded splenic biopsies were mixed with xylene, then ethanol, and the supernatant was then removed. The preparation was added to 180 µl of T1 buffer and 25 µl of proteinase K; the solution obtained was centrifuged and incubated at 56°C. Subsequently, 200 µl of B3 buffer were added and the solution was incubated at 70°C. 200 µl of ethanol were added and the solution was loaded on a NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) column. Two washes were performed using 500 µl of BW and B5 washing buffers. Last, 50 µl of BE buffer preheated to 70°C was used to collect the purified DNA, separating it from the filter in the column. To assess the quality of DNA, solutions were checked with a spectrophotometer at 230, 260, 280 and 320 nm.

For genetic identification of fungi and bacteria, DNA was prepared from paraffin slices using a previously established protocol followed by column purification using the High pure PCR purification kit (Roche Diagnostics, Rotkreuz, Switzerland).<sup>27</sup>

#### *PCR and sequencing*

PCR was used to assess the presence of microorganism in the spleen. Protocols used for bacterial and protozoan infections have been previously established,

whereas<sup>20,21,31</sup> the protocol for fungal PCRs was as follows: 15 min of denaturation at 95°C, 40 cycles of 94°C for 1 min, 51°C for 1 min, and 72°C for 1 min, and a 10 min final extension at 72°C. Amplified ribosomal DNA restriction analysis (ARDRA) was used to amplify the 16S rRNA of bacteria. Identification of bacteria was performed on a ~310 bp fragment of the 16S rRNA gene using primers 16SUNI-L and 16SRNAV-S and sequencing was performed according to a previously established protocol.<sup>20</sup>

Fungal internal transcribed spacer (ITSs), highly conserved RNA sequences located between two areas of ribosomal RNA, were amplified to detect the presence of mycotic agents.<sup>14</sup> In addition, identification of fungi was performed on a ~330 bp fragment of the internal transcribed spacer region 2 using primers ITS3 and ITS4.<sup>34</sup>

For protozoa, the analysis was performed to identify *Leishmania infantum* and *Hepatozoon* spp. The target sequence for *Leishmania* was LT1 of the conserved region of the circular kDNA; the primers used were RV1 (forward, 5'-CTTTTCTGGTCCCGCGGGTAGG-3') and RV2 (reverse, 5'-CCACCTGGCCTATTTTACACCA-3'), with a reported sensitivity of 100% in detecting *Leishmania* infections.<sup>21</sup> To detect the presence of *Hepatozoon* spp., a partial sequence of 18S rRNA, common to different species of *Hepatozoon*, was amplified. The primers used were HepF (forward, 5'-ATACATGAGCAAAATCTAAC-3') and HepR (reverse, 5'-CTTATTCCATGCTGCAG-3').<sup>31</sup>

All PCR products were submitted to electrophoresis in an 0.8% agarose gel and stained with ethidium bromide; an ultraviolet transilluminator was used to visualize the different bands.

### *Immunohistochemistry*

A monoclonal mouse anti-KMP11 antibody (dilution 1:300; Abcam, Cambridge, UK) was used to confirm the presence of *Leishmania infantum* in all PCR positive spleens to investigate the role of *Leishmania* in the pathogenesis of splenitis. Sections were incubated with the antibody for 24 min at room temperature. A canine splenic biopsy specimen previously proven to have *Leishmania* spp. amastigotes in macrophages served as a positive control. Immunohistochemistry was performed with an automatic immunostainer (Ventana Benchmark XT; Roche-Diagnostics, Monza, Italy) using a secondary antibody with a horseradish peroxidase (HRP)-conjugated polymer (ultraViews Universal DAB; Ventana Medical Systems, Tucson, AZ).

## Results

### *Dogs, clinical data, and questionnaire*

Thirty-three dogs were included in the study with a median age of 8.5 years (range: 2–15). Seventeen dogs were intact males (51.5%), 7 intact females (21.2%), 6 spayed females (18.2%), and 2 neutered males (6.0%); sex was unknown in the remaining dog. Ten dogs were mixed breed (30.3%) and 23 were pure breed dogs (69.7%), including 6 German Shepherds (18.2%), 2 each Great Dane (6.0%), Italian Hound (6.0%), Boxer (6.0%), Rottweiler (6.0%), and Labrador Retriever (6.0%), and 1 each English Bulldog (3.0%), English Setter (3.0%), Dachsbrake (3.0%), Griffon (3.0%), Airdale Terrier (3.0%), Doberman (3.0%), and Doberman Pinscher (3.0%).

According to the available clinical data and questionnaires, presenting complaints at the time of splenectomy or necropsy were known for 28 dogs. Severe and acute weakness was observed in 16 dogs (48.5%), hyperthermia in 11 (33.3%), weight loss in 6 (18.2%), and painful abdomen and vomiting in 4 (12.1%). Twenty-five animals underwent abdominal ultrasound that identified a splenic mass or nodules in 14 dogs (56.0%), peritoneal effusion in 8 (32.0%) consistent with purulent peritoneal exudate in 2, an altered echotexture of the spleen or a non-splenic abdominal mass in 4 each (16.0%), pyometra or endometritis, splenic torsion or testicular nodule in 3 dogs each (12.0%), portal vein and splenic vein thrombosis, prostatic nodules, hepatomegaly, and hypoechoic hepatic nodules in 1 dog each (4.0%). Twelve dogs presented more than one ultrasonographic alteration. In the remaining dogs, abdominal ultrasound was not performed because physical examination allowed diagnosis of gastric dilation-volvulus or an abdominal mass was palpated.

Among the 33 dogs, 24 (72.7%) were diagnosed with an additional non-splenic disease, including 8 (24.2%) with peritoneal effusion, 6 (18.2%) with neoplastic diseases (1 dog each with peritoneal hemangiosarcoma, testicular seminoma, fibrohistiocytic nodule, hepatic cystadenoma with concurrent testicular seminoma, metastatic mammary carcinoma, and hepatocellular carcinoma), 5 (15.1%) with gastric dilatation-volvulus, 3 (9.0%) with pyometra or endometritis, 3 (9.0%) with splenic torsions, 2 (6.0%) with granulomatous hepatitis (in 1 case due to leishmaniasis), 1 (3.0%) each with idiopathic hepatic lobar torsion, gastric ulcer, systemic fungal infection, immune-mediated hemolytic anemia, recent trauma, hemorrhagic gastroenteritis, colangio-hepatitis, and portal vein thrombosis. Eight dogs were concurrently affected by more diseases and 9 dogs (27.3%) did not show any disease other than splenitis.

Information on outcome was available for 19 dogs. The dog with systemic mycosis died a few days after admission and histological examination was performed as part of necropsy. The remaining 18 dogs underwent surgery and were then treated with different antibiotics; 16 (88.9%) survived to discharge, and 2 (11.1%) died during hospitalization because of surgical complications at 7 and 40 days after surgery, respectively. Of the 16 surviving dogs, 6 (37.5%) are still alive at the time of writing and 10 (62.5%) died because of causes unrelated to splenitis, with a median survival of 2.5 years (range: 1–3.5).

### *Histology*

The 33 cases of splenitis included in the analysis were classified histologically according to the criteria reported in Supplemental Table 1. In particular, 9 (27.3%)

dogs had purulent splenitis (figure 1), 8 (24.2%) pyogranulomatous splenitis (figure 2), 5 (15.1%) neutrophilic perisplenitis (figure 3), 4 (12.1%) neutrophilic perisplenitis and splenitis, 3 (9.0%) granulomatous splenitis (figure 4), 2 (6.0%) neutrophilic splenitis (figure 5), and 1 dog each (3.0%) with fibrin-suppurative perisplenitis and splenitis, and fibrin-suppurative perisplenitis (figure 6). In 1 dog affected by purulent splenitis and in 1 affected by pyogranulomatous splenitis, a splenic abscess was identified.

Of the 9 dogs with purulent splenitis, 6 yielded positive results with Gram staining (66.6%) (figure 1); in particular, it was possible to identify Gram-positive cocci in 3, Gram-negative cocci, concurrent Gram-positive cocci and bacilli, and concurrent Gram-negative and positive cocci in 1 dog each.

Among the 8 cases of pyogranulomatous splenitis, it was possible to identify meronts of *Hepatozoon canis* in 1 dog (figure 7a and figure 7b) and intra- and extra-cellular material compatible with exogenous material in another dog (figure 8). Among the 3 cases of granulomatous splenitis, it was possible to identify amastigotes of *Leishmania infantum* (figure 9) and fungal hyphae in 1 dog each (figure 10).

In total, 11 dogs presented histological signs of altered splenic circulation consistent with splenic hemorrhage, splenic hemotomas, splenic vein thromobosis, and splenic torsion; in particular, 4 dogs with neutrophilic perisplenitis and splenitis, 3 dogs with neutrophilic perisplenitis, 1 dog each with fibrinsuppurative perisplenitis and splenitis, granulomatous splenitis, fibrinsuppurative perisplenitis, and pyogranulomatous splenitis.

Twenty-four splenic samples yielded negative results for infectious agents at microscopic examination.

## PCR

Twenty-three dogs had positive results for bacterial DNA amplification (Supplemental Table 2); *Pseudomonas* spp. DNA was identified in 19 dogs, *Propionibacterium* spp. and *Clostridium hemolyticum* in 1 dog each, and mixed bacterial population in 2 dogs. Among these 23 dogs, histological diagnosis was consistent with neutrophilic or purulent-suppurative perisplenitis and splenitis in 13 dogs (56.5%), and granulomatous or pyogranulomatous splenitis in 9 dogs (39.1%); the 2 remaining dogs had fibrin-suppurative inflammation (8.7%).

Based on histology, bacterial infection was confirmed in only 3 of these 23 dogs: in particular, 1 Great Dane affected by gastric dilation-volvulus resulted positive for mixed bacterial infection at PCR analysis and histology identified Gram positive cocci, 1 Griffon with purulent peritoneal exudate and free abdominal gas presented *Clostridium haemolyticum* at PCR analysis while histology identified cocci and, finally, 1 Doberman Pinscher affected by endometritis resulted positive for *Pseudomonas* spp. infection at PCR analysis and histology identified concurrent Gram positive and negative cocci.

Of the 19 *Pseudomonas* spp. DNA-positive dogs, 8 resulted positive for a concurrent infection based on PCR analysis or histology: 4 dogs to *Leishmania infantum* and 4 to fungal infection (*Cryptococcus* spp. in 2 dogs, *Cladosporium* spp. and *Rasamsonia argillacea* in 1 dog each). One of the 2 dogs with mixed DNA bacterial population resulted positive for *Exophiala xenobiotica* DNA amplification. The DNA of *Truncatella angustata*, *Bipolaris cynodontis* and 1 not-previously cultured fungus were isolated in 1 dog each.



Overall, fungal DNA was amplified in 8 dogs (24.2%) and the corresponding histological diagnosis was consistent with purulent splenitis in 4, with granulomatous or pyogranulomatous splenitis in 3, and with neutrophilic perisplenitis in 1.

Six dogs (18.2%) were positive for *Leishmania infantum* DNA; 4 presented a concurrent infection. PCR identified *Pseudomonas* spp. DNA in 3 dogs and *Cladosporium* spp. in 1; histology identified Gram-positive cocci in 1 dog. The histological diagnosis suggested a neutrophilic perisplenitis in 2 dogs, and neutrophilic perisplenitis and splenitis, purulent splenitis, fibrin-suppurative, and pyogranulomatous splenitis in 1 dog each. None of these dogs was positive at immunohistochemistry for *Leishmania infantum* infection, and it was not possible to identify *Leishmania infantum* amastigotes at histological examination.

None of the dogs yielded positive results for *Hepatozoon* spp. using PCR.

## Discussion

To the authors' knowledge, this is the first comprehensive study on splenitis in dogs, a disease that has been scantily described so far. In the present series, 33 dogs had a histological diagnosis of splenitis, representing 5% of all submitted spleens. This prevalence is similar to that previously described by other authors and confirms that splenitis is uncommon in dogs.<sup>6,19</sup> Clinical and diagnostic findings of splenitis are unspecific or related to the associated disease and, therefore, its diagnosis relies upon histology.

Only 1 dog (3%) of the present series had no concurrent systemic disease along with splenitis and yielded negative results in all tests. Hence, the dog had a tentative diagnosis of primary splenitis. In 8 other dogs, although no obvious systemic disease was present, PCR and histology identified bacterial, fungal, or protozoan infection of the spleen. Indeed, in these dogs, PCR amplified *Pseudomonas* spp., a not previously cultured fungus, *Cladosporium* spp. or *Leishmania infantum* and histology allowed for observation of meronts of *Hepatozoon canis*. The actual role of the above microorganisms in the pathogenesis of splenitis remains unclear because systemic involvement was not identified. Thus, it cannot be excluded that in some the microorganism merely represented a passive bystander or contaminant, rather than the cause of splenitis.

Concurrent diseases were very frequent, being present in 24 cases (72.7%). For most, an explanation for splenitis was deemed possible. Six dogs had neoplastic diseases and might be considered to play a causative role in the development of splenitis. For instance, hemangiosarcoma and mammary adenocarcinoma have been shown to predispose towards thrombosis and to alter vascular anatomy, possibly

favoring bacterial engraftment.<sup>22</sup> In the present series, 2 dogs had these tumors and both had vascular splenic lesions detected together with splenitis. Two other dogs had a liver neoplasia; hepatic tumors have been sometimes associated with liver abscesses in dogs, likely due to increased tissue susceptibility to bacterial growth, which in turn might cause bacteremia and subsequent splenitis.<sup>43</sup> Five dogs presented gastric dilation-volvulus and all had splenitis along with splenic vascular abnormalities. We hypothesize that in these cases, as gastric dilatation-volvulus develops, normal splenic vascularity and blood supply is progressively compromised, thus generating splenic inflammation. In addition, bacterial translocation from the gut, which is observed in up to 43% of cases with gastric dilatation-volvulus,<sup>52</sup> might have had a primary role in the establishment of splenitis. In addition, splenic inflammation was likely associated with bacterial translocation in the 6 dogs affected by pyometra, endometritis, hemorrhagic gastroenteritis, gastric ulcer, purulent peritoneal effusion, and hepatic lobar torsion. *Propionibacterium* spp. DNA was amplified in 1 dog affected by pyometra; this bacterium has been previously associated with pyometra in a woman and was shown to translocate into the bloodstream in healthy dogs undergoing ovariohysterectomy.<sup>8,53</sup> It is remarkable that the blood reaching the spleen originates only from the arterial supply of the systemic circulation (i.e. splenic artery) and, differently from the liver, no venous blood from the gastrointestinal tract directly flows through the organ in dogs; hence, if pathogens translocate from the gut, to reach the spleen and cause splenitis they have to pass the liver, lungs, heart and eventually reach the systemic circulation.<sup>57</sup>

One dog presented laboratory signs compatible with intravascular hemolysis. This dog had a splenic torsion and was PCR-positive for *Leishmania infantum*; both conditions might have triggered splenitis due to altered vascularity and blood supply

or activation of the reticuloendothelial system, and likely caused anemia.<sup>32,40</sup> Five other dogs had granulomatous hepatitis with *Leishmania infantum* amastigotes or *Pseudomonas* spp., exogenous material, systemic *Rasamsonia argillacea*, and abdominal trauma; all of these conditions might have led to splenitis. A clear association between the concurrent disease and splenitis was not evident in only 3 dogs.

Histology allowed identification of the potential cause of splenitis in 10 dogs. Specifically, in 4 with pyogranulomatous or gramulomatous splenitis some exogenous material, fungal hyphae, *Hepatozoon canis*, and *Leishmania infantum* were identified. It is interesting to note that of the latter 3, PCR yielded *Rasamsonia argillacea* in the dog affected by systemic mycosis, whereas in the other 2 it did not amplify *Hepatozoon canis* or *Leishmania infantum* DNA. Searching for the 2 protozoa failed, probably because of the paucity of microorganisms in the sample. In 6 dogs with purulent splenitis, histology identified bacteria. Only 3 had bacterial DNA amplified with PCR, including 1 each with *Pseudomonas* spp., *Clostridium haemolyticum*, and a mixed population. In the remaining 23 dogs, showing a variety of histological patterns, no obvious cause was documented within the splenic parenchyma at microscopy. Therefore, based on these findings, histology is able to identify the cause of splenitis in only about one-third of cases.

Conversely, PCR amplified bacterial DNA in 23 dogs, but bacteria were observed by histology in only 3 cases, as already mentioned. It is therefore possible that PCR performed on paraffin-embedded splenic samples leads to various positive results due to its elevated sensitivity. However, some of the positive results might have been due to contamination of samples with environmental bacteria or to the presence of DNA fragments within macrophages and other cells of the spleen. The fact that 3

dogs had splenic bacteria based on histology, but not PCR, indicates that false-negative results can occur with the latter. It is possible that DNA damage caused by formalin fixation and paraffin embedding of samples also reduced the sensitivity of the assay. With regards to mycosis, in 8 dogs fungal DNA was found by PCR, but the presence of hyphae was confirmed by histology in only 1 case; the large majority (i.e. 7 of 8) presented neutrophilic or pyogranulomatous splenitis. The explanation for false positive or false negative PCR results for fungal infection is likely similar to those previously reported for bacteria. Concerning *Rasamsonia argillacea*, it is worth noting that this fungus is considered an emerging pathogen in humans, causing chronic granulomatous disease and cystic fibrosis.<sup>23,48</sup> *Rasamsonia argillacea* has been reported in only 2 dogs to date.<sup>12,37</sup> Other fungi identified in the present series were *Bipolaris cynodontis*, *Cladosporium spp.*, *Cryptococcus spp.*, *Exophiala xenobiotica*, *Truncatella angustata*, and an uncharacterized fungus. Among those that have never been described in dogs, *Bipolaris cynodontis* is a dematiaceous hyphomycetes widely diffuse in soil and normally associated with plant infection. It was recently associated with clinical disease in humans.<sup>29</sup> *Exophiala xenobiotica* is an opportunistic fungus responsible for phaeohyphomycosis, in particular in immune compromised humans.<sup>54</sup> *Truncatella angustata* is a coelomycetous fungus, recently reported to cause subcutaneous infection in an immune-competent man.<sup>18</sup> Since none has been reported in dogs, it cannot be excluded that they resulted from contamination of samples.

In relation to protozoa and PCR, 6 dogs yielded amplification of *Leishmania infantum* DNA and histological diagnosis was neutrophilic splenitis and perisplenitis in 3 cases, and fibrin-suppurative perisplenitis and splenitis, pyogranulomatous splenitis, and purulent splenitis with visible bacteria in 1 dog each. Except for the last case, similar

histological findings have been described in previous studies.<sup>35,38,49</sup> It is noteworthy that all PCR-positive dogs gave negative results with immunohistochemistry. It is thus possible that some splenic macrophages had phagocytosed DNA fragments, but that intact amastigotes were not detectable. The amplification methods used with PCR allow to obtain positive results with very small amounts of DNA, while this is not achievable by means of immunohistochemistry. Therefore, sensitivity of the former is expected to be superior, as demonstrated in a study that compared PCR and immunohistochemistry on paraffin-embedded canine skin biopsies for diagnosing leishmaniasis; the former yielded a sensitivity of 82.8% whereas the latter 62.1%.<sup>56</sup> None of the dogs was *Hepatozoon canis*-positive with PCR. The single dog with *Hepatozoon canis* based on histology did not display obvious clinical signs, perhaps because of the low parasitic burden.

In 19 dogs it was possible to collect data on outcome. Of these, 84.2% survived surgery and 36.8% are alive at the time of writing. The median survival of dogs that were followed-up until death was 2.5 years (range: 1–3.5); in these dogs, deaths were unrelated to the previous disease. The prognosis for dogs affected by splenitis can therefore be considered good.

There are some limitations to the present study that need to be mentioned. Because of the retrospective nature of the investigation and the fact the cases came from different institutions, information gathered from available medical records were often incomplete. In addition, samples were retrieved from archives and prior contamination cannot be excluded.

## Conclusions

In conclusion, splenitis is an uncommon disease in dogs; clinical signs are vague and concurrent diseases that might predispose to splenic inflammation are generally present. Prognosis is favorable in the majority of cases. Identification of bacteria, fungi, and protozoa in the spleen of dogs with splenitis should be interpreted cautiously if PCR is used, since histology does not provide supportive evidence of infection in many cases. Furthermore, the histologic classification used in the present study is expected to be helpful for future investigations, either for clinicians or pathologists.

### **Acknowledgements**

The authors are grateful to Dr. Rossella Zanetti and Dr. Emilia Cavicchioli for excellent technical support.

### **Declaration of Conflicting Interests**

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## References

1. Abdellatif A, Günther C, Peppler C, Kramer M. A rare case of splenic abscess with septic peritonitis in a German shepherd dog. *BMC Vet Res.* 2014;10:201.
2. Ballegeer EA, Forrest LJ, Dickinson RM, Schutten MM, Delaney FA, Young KM. Correlation of ultrasonographic appearance of lesions and cytologic and histologic diagnoses in splenic aspirates from dogs and cats: 32 cases (2002-2005). *J Am Vet Med Assoc.* 2007;230:690-696.
3. Baneth G, Weigler B. Retrospective case-control study of hepatozoonosis in dogs in Israel. *J Vet Intern Med.* 1997;11(6):365-370.
4. Brown MR, Thompson CA, Mohamed FM. Systemic candidiasis in an apparently immunocompetent dog. *J Vet Diagn Invest.* 2005;17(3):272-276.
5. Campora L, Corazza M, Zullino C, Ebani VV, Abramo F. *Mycobacterium avium* subspecies *hominissuis* disseminated infection in a Basset Hound dog. *J Vet Diagn Invest.* 2011;23:1083-1087.
6. Christensen N, Canfield P, Martin P, Krockenberger M, Spielman D, Bosward K. Cytopathological and histopathological diagnosis of canine splenic disorders. *Aust Vet J.* 2009;87:175-181.
7. Collett MG, Doyle AS, Reyers F, Kruse T, Fabian B. Fatal disseminated cryptococcosis and concurrent ehrlichiosis in a dog. *J S Afr Vet Assoc.* 1987;58:197-202.
8. Dahlinger J, Marks SL, Hirsh DC. Prevalence and identity of translocating bacteria in healthy dogs. *J Vet Intern Med.* 1997;11(6):319-322.
9. Day MJ, Lucke VM, Pearson H. A review of pathological diagnoses made from 87 canine splenic biopsies. *J Small Anim Pract.* 1995;36:426-433.



10. Eberle N, von Babo V, Nolte I, Baumgärtner W, Betz D. Splenic masses in dogs. Part 1: Epidemiologic, clinical characteristics as well as histopathologic diagnosis in 249 cases (2000-2011). *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2012;40:250-260.
11. Ginel PJ, Lucena R, Arola J, Martin MP, Mozos E. Diffuse splenomegaly caused by splenic abscessation in a dog. *Vet Rec*. 2001;149:327-329.
12. Grant DC, Sutton DA, Sandberg CA, et al. Disseminated *Geosmithia argillacea* infection in a German shepherd dog. *Med Mycol*. 2009;47(2):221-226.
13. Haynes SM, Hodge PJ, Tyrrell D, Abraham LA. Disseminated *Scedosporium prolificans* infection in a German Shepherd dog. *Aust Vet J*. 2012;90:34-38.
14. Hendolin PH, Paulin L, Koukila-Kähkölä P, et al. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J Clin Microbiol*. 2000;38:4186-4192.
15. Hervàs J, Carrasco L, Gòmez-Villamandos JC, Méndez A, Sierra MA. Acute fatal hepatozoonosis in a puppy: histopathological and ultrastructural study. *Vet Rec*. 1995;137:518-519.
16. Horn B, Forshaw D, Cousins D, Irwin PJ. Disseminated *Mycobacterium avium* infection in a dog with chronic diarrhoea. *Aust Vet J*. 2000;78:320-325.
17. Hoskins JD, Bunge MM, Dubey JP, Duncan D.E. Disseminated infection with *Neospora caninum* in a ten-year-old dog. *Cornell Vet*. 1991;81:329-334.
18. Jagielski T, Żak I, Tyrak J, Bryk A. First Probable Case of Subcutaneous Infection Due to *Truncatella angustata*: a New Fungal Pathogen of Humans? *J Clin Microbiol*. 2015;53(6):1961-1964.

19. Johnson KA, Powers BE, Withrow SJ, Sheetz MJ, Curtis CR, Wrigley RH. Splenomegaly in dogs. Predictors of neoplasia and survival after splenectomy. *J Vet Intern Med.* 1989;3:160-166.
20. Kuhnert P, Frey J, Lang NP, Mayfield L. Phylogenetic analysis of *Prevotella nigrescens*, *Prevotella intermedia* and *Porphyromonas gingivalis* clinical strains reveals a clear species clustering. *Int J Syst Evol Microbiol.* 2002;52:1391-1395.
21. Lachaud L, Marchergui-Hammami S, Chabbert E, Dereure J, Dedet JP, Bastien P. Comparison of six PCR methods using peripheral blood for detection of canine visceral leishmaniasis. *J Clin Microbiol.* 2002;40:210-215.
22. Laurenson MP, Hopper K, Herrera MA, Johnson EG. Concurrent diseases and conditions in dogs with splenic vein thrombosis. *J Vet Intern Med.* 2010;24:1298-1304.
23. Machouart M, Garcia-Hermoso D, Rivier A, et al. Emergence of disseminated infections due to *Geosmithia argillacea* in patients with chronic granulomatous disease receiving long-term azole antifungal prophylaxis. *J Clin Microbiol.* 2011;49(4):1681-1683.
24. Mackie JT, Padhye AA, Sutherland RJ, Lamb WA, Davis S. Granulomatous lymphadenitis and splenitis associated with *Monocillium indicum* infection in a dog. *J Vet Diagn Invest.* 2004;16:248-250.
25. Martinho AP, Franco MM, Ribeiro MG, et al. Disseminated *Mycobacterium tuberculosis* infection in a dog. *Am J Trop Med Hyg.* 2013;88(3):596-600.
26. McGee ED, Fritz DL, Ezzell JW, Newcomb HL, Brown RJ, Jaax NK. Anthrax in a dog. *Vet Pathol.* 1994;31(4):471-473.
27. Müller N, Zimmermann V, Forster U, Bienz M, Gottstein B, Welle M. PCR-based detection of canine *Leishmania* infections in formalin-fixed and paraffin-

- embedded skin biopsies: elaboration of a protocol for quality assessment of the diagnostic amplification reaction. *Vet Parasitol.* 2003; 114(3): 223-229.
28. Nabity MB, Barnhart K, Logan KS, et al. An atypical case of *Trypanosoma cruzi* infection in a young English Mastiff. *Vet Parasitol.* 2006;140(3-4):356-361.
29. Nath R, Barua S, Barman J, Swargiary P, Borgohain M, Saikia L. Subcutaneous Mycosis Due to *Cladosporium cladosporioides* and *Bipolaris cynodontis* from Assam, North-East India and Review of Published Literature. *Mycopathologia.* 2015;180(5-6):379-387.
30. O'Toole D, Tharp S, Thomsen BV, Tan E, Payeur JB. Fatal mycobacteriosis with hepatosplenomegaly in a young dog due to *Mycobacterium avium*. *J Vet Diagn Invest.* 2005;17:200-204.
31. Otranto D, Dantas-Torres F, Weigl S, et al. Diagnosis of *Hepatozoon canis* in young dogs by cytology and PCR. *Parasit Vectors.* 2011;4:55.
32. Paltrinieri S, Solano-Gallego L, Fondati A, et al. Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. *J Am Vet Med Assoc.* 2010;236(11):1184-1191.
33. Pastor J, Pumarola M, Cuenca R, Lavin S. Systemic aspergillosis in a dog. *Vet Rec.* 1993;132(16):412-413.
34. Rampazzo A, Kuhnert P, Howard J, Bornand V. *Hormographiella aspergillata* keratomycosis in a dog. *Vet Ophthalmol.* 2009 12:43-47.
35. Reis AB, Teixeira-Carvalho A, Giunchetti RC, et al. Cellular immunophenotypic profile in the splenic compartment during canine visceral leishmaniasis. *Vet Immunol Immunopathol.* 2014;157:190-196.

36. Richardson EF, Brown NO. Hematological and biochemical changes and results of aerobic bacteriological culturing in dogs undergoing splenectomy. *J Am Anim Hosp Assoc.* 1996;32:199-210.
37. Salgüero R, Borman AM, Herrtage M, et al. *Rasamsonia argillacea* mycosis in a dog: first case in Europe. *Vet Rec.* 2013;172(22):581.
38. Santana CC, Vassallo J, De Freitas LAR, Oliveira GG, Pontes-de-Carvalho LC, dos-Santos WL. Inflammation and structural changes of splenic lymphoid tissue in visceral leishmaniasis: A study on naturally infected dogs. *Parasite Immunology.* 2008;30:515-524.
39. Saunders GK, Monroe WE. Systemic granulomatous disease and sialometaplasia in a dog with *Bartonella* infection. *Vet Pathol.* 2006;43:391-392.
40. Schnier LM. A case of splenic torsion with progressive anemia and thrombocytopenia. *Can Vet J.* 2010;51:527-529.
41. Schroeder H, van Rensburg IB. Generalised *Listeria monocytogenes* infection in a dog. *J S Afr Vet Assoc.* 1993;64:133-136.
42. Schroeder H, Jardine JE, Davis V. Systemic phaeohyphomycosis caused by *Xylohypha bantiana* in a dog. *J S Afr Vet Assoc.* 1994;65:175-178.
43. Schwarz LA, Penninck DG, Leveille-Webster C. Hepatic abscesses in 13 dogs: a review of the ultrasonographic findings, clinical data and therapeutic options. *Vet Radiol Ultrasound.* 1998;39(4):357-365.
44. Simpson KW, Khan KN, Podell M, Johnson SE, Wilkie DA. Systemic mycosis caused by *Acremonium* spp. in a dog. *J Am Vet Med Assoc.* 1993;203:1296-1299.

45. Singh K, Flood J, Welsh RD, Wyckoff JH, Snider TA, Sutton DA. Fatal systemic phaeohyphomycosis caused by *Ochroconis gallopavum* in a dog (*Canis familiaris*). *Vet Pathol*. 2006;43:988-992.
46. Spangler WL, Culbertson MR. Prevalence, type, and importance of splenic diseases in dogs: 1,480 cases (1985-1989). *J Am Vet Med Assoc*. 1992;200:829-834.
47. Stedham MA. Histopathology of melioidosis in the dog. *Lab invest*. 1977;36:358.
48. Steinmann J, Giraud S, Schmidt D, et al. Validation of a novel real-time PCR for detecting *Rasamsonia argillacea* species complex in respiratory secretions from cystic fibrosis patients. *New Microbes New Infect*. 2014; 2(3): 72-78.
49. Tasca KI, Buzetti WA, Tenorio Mda S, et al. Parasitological, immunohistochemical and histopathological study for *Leishmania chagasi* detection in splenic tissues of dogs with visceral leishmaniasis. *Rev Bras Parasitol Vet*. 2009;18:27-33.
50. Tizard IR. "Organs of the immune system". In: Tizard IR. *Veterinary immunology* 9th ed. St. Louis, Missouri: Elsevier Saunders; 2013: 124-126
51. Welsh RD, Ray WE. *Scopulariopsis chartarum* Systemic Mycosis in a Dog. *J Clin Microbiol*. 1999; 37: 2102–2103.
52. Winkler KP, Greenfield CL, Schaeffer DJ. Bacteremia and bacterial translocation in the naturally occurring canine gastric dilatation-volvulus patient. *J Am Anim Hosp Assoc*. 2003;39(4):361-368.
53. Yamada E, Ueda T, Mori A, Haraguchi H, Nagata Y. Clinical experience with aztreonam in the field of obstetrics and gynecology. *Jpn J Antibiot*. 1985;38(12):3695-3699.

54. Zeng JS, Sutton DA, Fothergill AW, Rinaldi MG, Harrak MJ, Hoog GS. Spectrum of Clinically Relevant *Exophiala* Species in the United States. *J Clin Microbiol.* 2007;45(11):3713-3720.
55. Mauricio IL, Howard MK, Stothard JR, Miles MA. Genomic diversity in the *Leishmania donovani* complex. *Parassitology.* 1999;119(3):237-246.
56. Xavier SC, de Andrade HM, Monte SJ, et al. Comparison of paraffin-embedded skin biopsies from different anatomical regions as sampling methods for detection of *Leishmania* infection in dogs using histological, immunohistochemical and PCR methods. *BMC Vet Res.* 2006;2:17.
57. Greene CE, Weese JS. "Surgical and Traumatic Wound Infections". In: Greene CE. Infectious disease of the dog and cat 4th ed. St. Louis, Missouri: Elsevier Saunders; 2012:565.

## Figure legend

Figures 1-6. Spleen, dog. Hematoxylin and Eosin. Figure 1. Purulent splenitis.

Presence of a purulent infiltrate. Upper inset: Higher magnification of the infiltrate shows degenerated neutrophils and macrophages. Lower inset: blue-stained coccoid

bacteria. GRAM. Figure 2. Pyogranulomatous splenitis. A granuloma with

macrophages and neutrophils. Figure 3. Neutrophilic perisplenitis. The capsule is infiltrated by neutrophils and the mesothelium appears reactive and hyperplastic.

Figure 4. Granulomatous splenitis. Nodular aggregate of macrophages, lymphocytes, and plasmacells. Inset: Higher magnification. Figure 5. Neutrophilic splenitis.

Numerous non-degenerated, non-lytic neutrophils invade the splenic parenchyma.

Figure 6. Fibrinous perisplenitis. Organized fibrin as pseudomembranous deposit on the capsular surface.

Figures 7-10. Spleen, dog. Figure 7a. A round meront in the splenic parenchyma with “weehl spoke” arrangement of the merozoites. Hematoxylin and eosin (HE). Figure 7b.

The merozoites stain strongly, and the capsule is negative. Periodic-acid Schiff (PAS).

Figure 8. Crystalloid three-dimensional acellular material in the center of a

pyogranuloma. Figure 9. Leishmaniosis. A granuloma composed of macrophages

and lymphoplasmacytic infiltrate. The arrow shows some very small intracytoplasmic vacuoles containing amastigotes. HE. Inset: Immunolabeling of intracytoplasmic

amastigotes in macrophages. Immunohistochemistry for KMP11. Figure 10. A

granuloma containing numerous branching hyphae. PAS.